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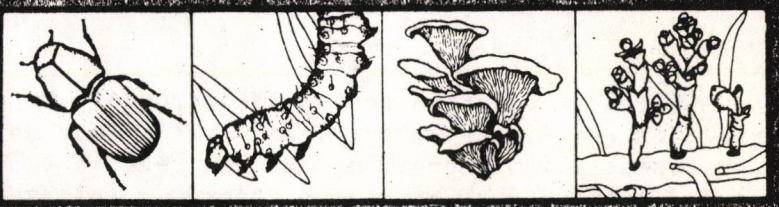

Report 84-14 December 1984

STUDIES OF FUSARIUM ASSOCIATED WITH CONTAINERIZED CONIFER SEEDLING DISEASES: PATHOGENICITY TESTS OF ISOLATES FROM THE ALPINE NURSERY, KALISPELL, MONTANA

by

R. L. James, Plant Pathologist, and C. J. Gilligan, Biological Technician

ABSTRACT

Isolates of Fusarium oxysporum, F. acuminatum, and F. sambucinum obtained from peat-vermiculite soil mixes were tested for pathogenicity on ponderosa pine, lodgepole pine, western larch, and blue spruce containerized seedlings and on germination of Scots pine and blue spruce seed. Ponderosa pine seedling were the least susceptible to killing by the Fusarium isolates tested. Fusarium acuminatum was the most pathogenic and F. sambucinum the least pathogenic on seedlings. Isolates of F. oxysporum ranged in virulence from very low to moderately high. The only isolate that consistently reduced seed germination was F. acuminatum. Time required for germination was generally not affected by the Fusarium isolate tested. Seedling inoculation techniques allowed for successful differentiation of the pathogenic potential of the Fusarium isolates tested.

INTRODUCTION

Diseases caused by Fusarium spp. are common on containerized conifer seedlings grown in greenhouses. Five different types of disease associated with Fusarium spp. are generally recognized. They include (1) seed decay (James 1983; James 1984b), (2) pre-emergence damping-off or germination failure (Bloomberg 1971; Filer and Peterson 1975), (3) post-emergence damping-off (Filer and Peterson 1975; Tint 1945a), (4) top damping-off or cotyledon blight (Bloomberg 1971; James 1984c), and (5) root disease or late damping-off (Lock 1973; Salisbury 1954; Smith 1975). Losses of containerized stock are often dependent on amount of natural seed infection (James 1984b; James 1984d), as well as characteristics of the growing medium (James 1984a, Pawuk 1978; Pawuk 1981) and conditions under which seedlings are grown (Landis 1976; Pawuk 1978).

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Severe losses occurred to the 1983 crop of containerized conifer seedlings grown at the Alpine Nursery, Kalispell, Montana. Species of Fusarium were consistently isolated from diseased seedlings. Affected seedlings were stunted, often with extensive chlorotic foliage. Many diseased seedlings died within a few weeks of developing symptoms. The most severely damaged species included Engelmann spruce (Picea engelmanii Parry), blue spruce (Picea pungens Engelm.), western larch (Larix occidentalis Nutt.) and lodgepole pine (Pinus contorta Dougl). Ponderosa pine (Pinus ponderosa Laws.) and Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) were also affected, but at a lower level.

The severity of disease encountered during 1983 had not previously occurred at the nursery. No alterations in growing regimes were used during 1983. The only major change was use of a different growing medium (soil mix). The soil mix used was a standard peat-vermiculite mixture, but was obtained from a different manufacturer than previously used. Nutrient and pH analyses of the new soil mix indicated that normal nutrient levels were present, but pH values were abnormally high (6.0-6.7).

Because of the consistent association of pathogenic fungi with diseased seedlings, soil mix samples were evaluated for presence of two common nursery pathogens, Fusarium and Pythium. Analyses indicated relatively high populations of Fusarium (avg. = 440 propagules per gram of tested soil mix (ppg) and Pythium (avg. = 150 ppg) (D. Reynolds, personal communication).

Occurrence of potentially pathogenic fungi in the soil mix does not necessarily implicate the soil mix as the source of disease. The pathogenic potential of these fungi should be investigated in order to evaluate their role in causing disease. Since species and/or races of Fusarium may be saprophytic as well as pathogenic (Armstrong and Armstrong 1975; Gordon 1965; Tint 1945a; Tint 1945b), it is important to test associated isolates for pathogenicity. This will help elucidate possible roles of isolated fungi in causing the observed disease. Therefore, isolates of Fusarium associated with the disease were characterized and used in pathogenicity tests to clarify their disease roles and to determine possible susceptibility differences. If consistent host susceptibility differences existed, occurrence of formae specialis within specific taxa could be confirmed (Booth 1975; Iannelli et al. 1982).

MATERIALS AND METHODS

Fusarium Isolation and Characterization - Four soil mix samples were collected from greenhouse benches (diseased seedlings were located on sampled benches). Another soil mix sample was obtained from a stunted, nonchlorotic Engelmann spruce seedling. One additional sample was collected from greenhouse floor soil to determine if it might contain pathogenic fungi.

Samples were prepared for special dilutions to determine relative abundance of Fusarium[1] (measured as ppg) (Nash and Snyder 1962). Samples were air dried for 7 days, then sieved through a 48-mesh (0.295 mm) screen; 0.025 g of sieved

[1]Investigations were limited to Fusarium spp. becasue of their common association with diseased seedlings at the Alpine Nursery and widespread occurrence on containerized conifer seedlings elsewhere. The levels of Pythium isolated from soil mixes were also sufficiently high to warrant concern.

soil were suspended in 10 ml of 0.1 percent water agar. One ml of the suspension was placed on selective agar media for Fusarium (Komada 1975). Four replicates of each soil sample were plated on the selective media. Plates were incubated under 12-hour diurnal cycles of cool fluorescent light at about 22°C for 7 days. After incubation, colonies of Fusarium were counted (each colony was assumed to arise from one fungal propagule).

Selected Fusarium isolates were transferred to and maintained on potato dextrose agar (PDA) for characterization and pathogenicity tests. The most prevalent isolate from each soil sample was transferred. Five isolates were obtained from soil mixes and two from necrotic roots of the Engelmann spruce seedling incubated on Komada's selective medium (table 1). Isolates were characterized using criteria of Booth (1971) and Gerlach and Nirenberg (1982). Taxonomic classifications were tentative and required confirmation.

Pathogenicity Tests - The seven Fusarium isolates were tested for their ability to attack and cause disease symptoms on 8-week-old containerized conifer seedlings. Four host species (ponderosa pine, lodgepole pine, western larch, and blue spruce) were evaluated in these tests. Additional tests were conducted on Scots pine (P. sylvestris L.) and blue spruce to evaluate ability of test isolates to cause pre-emergence and "early" post-emergence damping-off.

Inoculum was prepared as described by Miles and Wilcoxson (1984). Inoculum was produced in galvanized metal pans (5 x 25 x 35 cm) lined with a double layer of aluminum foil. Perlite, an inert, inorganic, siliceous rock of volcanic origin commonly used in potting mixtures, was the matrix for fungal growth. In each metal pan, 150 g of yellow cornmeal was moistened with 300 ml warm 1 percent PDA and left standing for 15 minutes, then 75 g of perlite was thoroughly mixed with the cornmeal. The pans were covered with aluminum foil and autoclaved for 60 minutes at 121°C. After cooling, the perlite-cornmeal cake was inoculated with 1 cm square pieces of mycelium from 14-day-old Fusarium cultures grown on PDA. Pieces from two culture plates were mixed with the perlite-cornmeal cake with a sterile knife. Fifty ml of sterile distilled water was added after mixing and the pan was sealed. Closed pans were incubated in the dark at about 24°C for 24 days. Fungal cake mixtures were air dried on a tabletop for 3 days and stored in plastic bags at 8°C until needed.

Table 1.--Population counts of Fusarium isolates obtained from the Alpine Nursery.

Isolate designation	Sample description	Population counts (PPG)[1]
84-21A	Engelmann spruce seedling roots	-
84-21B	Engelmann spruce seedling roots	-
84-22A	Soil mix from benches 8 and 9	100
84-22B	Soil mix from benches 3 and 4	200
84-22C	Soil mix from bench 2	200
84-22D	Soil mix from benches 5 and 6	600
84-22F	Soil mix from Engelmann spruce seedling	600

[1]Obtained from serial dilutions monitored on Komada's medium (Komada 1975).

In the first test, 10 seedlings of each host species were inoculated with each of the seven Fusarium isolates. Seedlings were carefully removed from their styroblock containers to minimize damage to young roots. Excess soil mix was carefully rinsed from roots and seedlings were repotted into clean styroblock containers (90 cm^3 capacity) using approximately 10 g of an inoculum-sterilized soil mixture. This growing medium consisted of 5 g of inoculum thoroughly mixed with 100 g (1:20 mixture) of sterilized (121°C for 60 minutes) Terra-lite Forestry Mix® (W. R. Grace and Co.), a peat-vermiculite growing medium designed for conifer seedlings. Noninoculated perlite mixed with sterilized Forestry Mix® served as a control.

In the second test, clean styroblock containers were filled with about 10 g of the inoculum-sterilized soil mixture (1:20 mixture). Ten cavities were inoculated with each test isolate and 10 uninoculated (perlite-sterilized soil mixture) cavities served as controls. Three seeds of either Scots pine or blue spruce were placed in each cavity and covered with about 1 cm of perlite. One hundred untreated, stratified seeds of each species were incubated on Komada's medium to determine levels of natural Fusarium infection (James 1983; James 1984b). Percentage infected with Fusarium, common saprophytic fungi (Trichoderma and Penicillium), and unidentified bacteria were determined.

At the time of inoculation, a sample of the inoculum-sterilized soil mixture was analyzed for inoculum density. Standard soil dilution techniques previously described were used to estimate ppg for each test isolate.

Inoculated seedlings were incubated within growth chambers at about 24°C under 12-hour diurnal cycles of cool fluorescent light. Seedlings were not fertilized, were watered about three times a week, and were periodically examined for disease symptoms (general wilting and necrosis, chlorosis, leader crook, and needle tip dieback). Symptom progression was monitored over time. Isolations were made from the roots of killed seedlings onto Komada's medium to verify pathogenicity of inoculated isolates. In addition to Fusarium, occurrence of two common saprophytic fungi, Trichoderma and Penicillium were assayed. Relative numbers of these saprophytes on roots may influence colonization by pathogens, such as Fusarium. Isolations were also made from roots of seedlings without disease symptoms at the end of the experiment (68-80 days from inoculation). For these isolations, a minimum of 15 fine root tips were randomly selected from throughout each seedling's root system. Although not precisely quantitative, this sampling technique gave a general estimate of the extent of each root system colonized by Fusarium.

For the seed germination-damping-off test, inoculated seed were incubated in growth chambers as described above. Seed germination and germling necrosis (damping-off) were monitored over time. When germlings were killed, they were incubated on Komada's medium to verify pathogenicity of inoculated isolates. Isolations were also made from roots of germlings that remained alive throughout the experiment (68 days). Percentage seed germination, average time required for germination, percent germling mortality and average survival time of germlings were calculated for each test isolate.

RESULTS

Fusarium Isolation and Characterization - Three separate species of Fusarium[2] were isolated from soil mix samples and spruce seedling roots. Descriptions of all isolates are detailed in the Appendix.

The most prevalent species encountered was F. oxysporum Schlect. Five of the seven Fusarium isolates obtained for pathogenicity tests were classified as this species. All the F. oxysporum isolates obtained produced purple pigmentation in culture. However, isolate differences were noted on the basis of other cultural characteristics such as growth rate, maintenance of floccose mycelium, production of sporodochia and sclerotia, and relative abundance of different spore types, especially chlamydospores.

The other isolated Fusarium species were F. acuminatum Ell. and Kellerm. (= F. roseum Link emend. Snyd. & Hansen) (isolate 84-22A) and F. sambucinum Fuckel. (= F. roseum) (isolate 84-21A). These species were found less frequently than F. oxysporum.

Pathogenicity Tests - Effects of the Fusarium isolates on seedling mortality are summarized in table 2. Differences in species susceptibility as well as isolate virulence were evident. Ponderosa pine was less susceptible to killing than either lodgepole pine, western larch, or blue spruce. We also found that isolate 84-22A (F. acuminatum) was the most pathogenic and 84-21A (F. sambucinum) the least pathogenic on these seedlings. Isolates of F. oxysporum ranged in virulence from very low (84-21B) to moderately high (84-22D).

Effects of Fusarium isolates on production of foliage symptoms are summarized for ponderosa pine and lodgepole pine in table 3 and western larch and blue spruce in table 4. Symptoms ranged from slight needle tip necrosis to extensive foliar wilting and necrosis. Wilting was often accompanied by needle twisting and crooking or bending of the leader (figure 1). Severity of foliage symptoms generally corresponded to relative virulence levels of isolates as evidenced by seedling death.

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[2]Identification of the species of Fusarium was based on taxonomic schemes described by Booth (1971) and Gerlach and Nirenberg (1982). Identifications were confirmed by S. J. Cooley (USDA For. Serv., Portland, OR).

Table 2.--Effects of Fusarium isolates on mortality of containerized ponderosa pine, lodgepole pine, western larch, and blue spruce seedlings.

	Percentage seedlings killed[1]					
Isolate	Ponderosa pine	Lodgepole pine	Western larch	Blue spruce	All species	All species but ponderosa pine
84-21A	0	10	10	10	7.5	10.0
84-21B	0	30	10	40	20.0	26.7
84-22A	0	100	100	90	72.5	96.7
84-22B	40	60	100	50	62.5	70.0
84-22C	50	60	40	30	45.0	43.3
84-22D	10	70	80	70	57.5	73.3
84-22F	0	60	60	40	40.0	53.3
All inoculated	14.3	55.7	57.1	47.1	43.6	53.3
Control	0	10	10	0	50	6.7

[1]Within 80, 69, 68, and 68 days of inoculation for ponderosa pine, lodgepole pine, western larch, and blue spruce, respectively.

Table 3.--Effects of Fusarium isolates on the production of foliage symptoms by containerized ponderosa and lodgepole pine seedlings

	Percent seedlings with symptoms[1]							
	Ponderosa pine[2] Symptom class				Lodgepole pine[2] Symptom class			
Isolate	1	2	3	4	1	2	3	4
84-21A	40	50	0	10	40	0	10	50
84-21B	30	60	0	10	20	0	30	50
84-22A	40	50	0	10	0	0	10	0
84-22B	10	50	40	0	10	0	60	30
84-22C	0	50	50	0	10	20	60	10
84-22D	20	60	10	10	10	0	70	20
84-22F	20	70	0	10	20	0	60	20
All inoculated	23.7	50.0	12.5	13.8	17.5	2.5	50.0	30.0
Control	30	10	0	60	30	0	10	60

[1]Symptoms at time of seedling death or the end of the experiment (80 and 69 days for ponderosa and lodgepole pine, respectively).

[2]Symptom classes:

1 = Slight needle tip necrosis
2 = Necrotic needles at the base of seedling
3 = Extensive foliar wilting and/or necrosis
4 = No foliage symptoms

Table 4.--Effects of Fusarium isolates on the production of foliage symptoms by containerized western larch and blue spruce seedlings.

	Percent seedlings with symptoms[1]							
	Western larch[2] Symptom class				Blue spruce[2] Symptom class			
Isolate	1	2	3	4	1	2	3	4
84-21A	0	60	10	30	10	40	10	40
84-21B	0	50	10	40	20	0	40	40
84-22A	0	0	100	0	10	0	90	0
84-22B	0	0	100	0	0	10	50	40
84-22C	0	30	40	30	20	0	30	50
84-22D	0	20	80	0	30	0	70	0
84-22F	10	20	60	10	20	0	40	40
All inoculated	1.3	25.0	51.2	22.5	15.0	7.5	41.3	36.2
Control	0	20	10	70	10	10	0	80

[1]Symptoms at time of seedling death or the end of the experiment (68 days).
[2]Symptom classes:
1 = Slight needle tip necrosis
2 = Necrotic needles at the base of seedling
3 = Extensive foliar wilting and/or necrosis
4 = No foliage symptoms

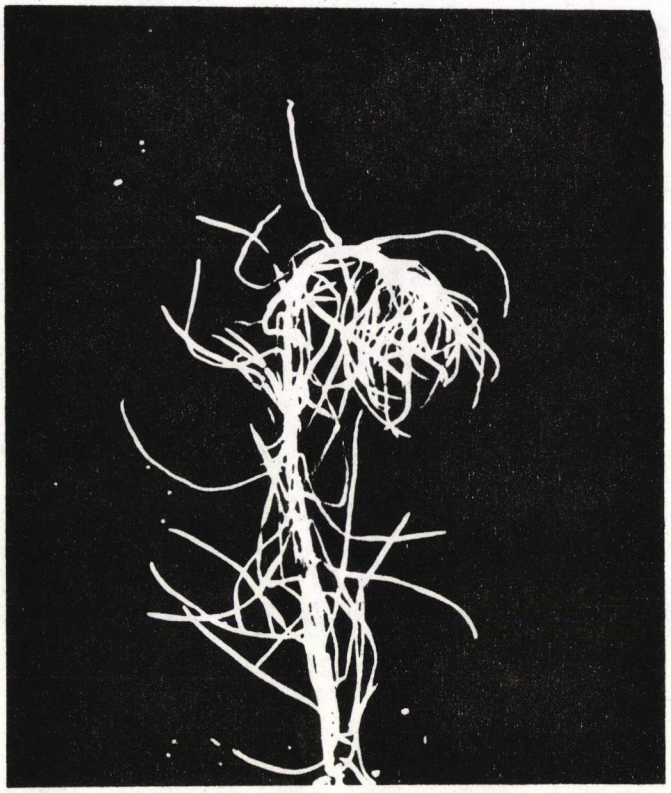

Figure 1.--Lodgepole pine seedling killed by isolate 84-22A F. acuminatum). Wilting symptoms were accompanied by needle twisting and crooking of the leader.

Tables 5-9 summarize reisolation results from roots of inoculated seedlings. Values in tables are expressed as percentages of root samples which yielded the inoculated Fusarium isolate, other Fusarium isolates, and two common saprophytic genera, Trichoderma and Penicillium. Inoculated isolates of Fusarium were reisolated from all seedlings, i.e., at least a few of the sampled roots yielded the inoculated isolate. This occurred for both dead and live seedlings, although percentage of recovery tended to be higher for dead seedlings (figure 2). Average recovery of the inoculated isolate was about 97 percent for seedlings killed by that isolate and 77 percent for seedlings not killed (table 9). Isolation of Trichoderma and Penicillium species were also common for most treatments.

Species of Fusarium other than the inoculated isolate, were sometimes obtained from seedling roots (tables 5-9). This was most common on seedlings inoculated with isolates 84-21A and 84-22A. In both cases, F. oxysporum was sometimes recovered from the roots of these seedlings. Also, several control seedlings (noninoculated) yielded Fusarium on their roots. This may have been due to inoculum spread among treatments during the experiment, since treatments were often located close together (within adjacent rows in styroblock containers).

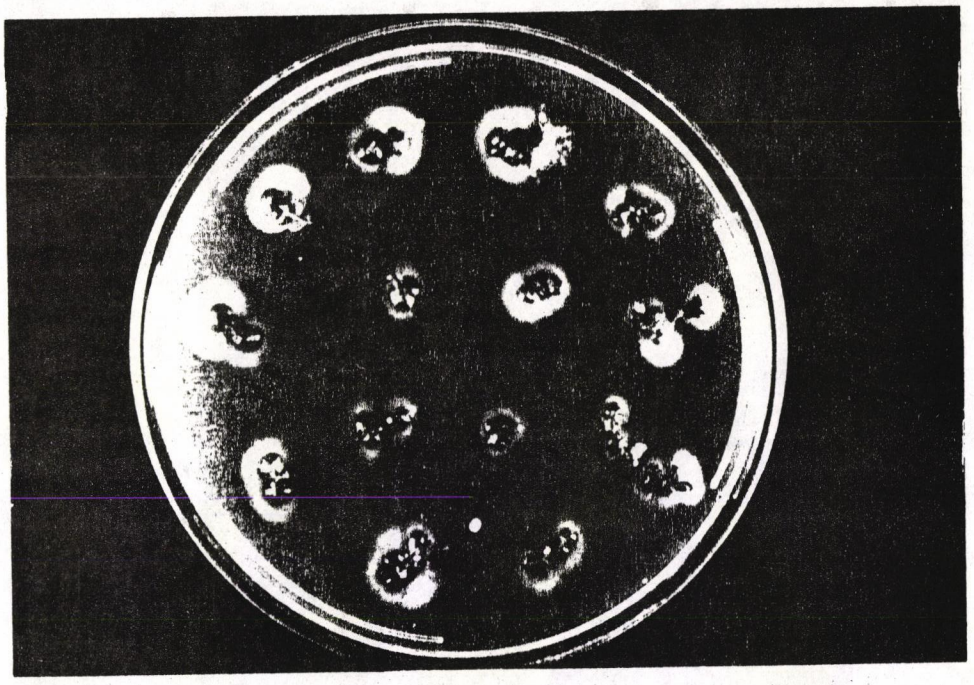

Figure 2.--Reisolation of Fusarium oxysporum (isolate 84-21B) from the roots of a western larch seedling killed by this isolate. All root samples on this plate yielded the isolate when incubated on Komada's medium for 7 days at 22°C.

Table 5.—Recovery of fungi from inoculated roots of ponderosa pine seedlings.

	PERCENTAGE OF ROOT SAMPLES												
	Dead seedlings				Live seedlings				All seedlings				
Isolate	Inoc[1]	Other[2] FUS	TRI[3]	PEN[4]	Inoc[1]	Other[2] FUS	TRI[3]	PEN[4]	Inoc[1]	Other[2] FUS	TRI[3]	PEN[4]	No. root samples
84-21A	-	-	-	-	35.3	23.3	11.3	71.3	35.3	23.3	11.3	71.3	150
84-21B	-	-	-	-	59.3	12.0	6.7	82.7	59.3	12.0	6.7	82.7	150
84-22A	-	-	-	-	82.0	14.0	74.7	87.3	82.0	14.0	74.7	87.3	150
84-22B	93.3	0	33.3	48.3	90.1	6.6	1.1	53.9	91.4	4.0	13.9	51.7	151
84-22C	100.0	0	41.0	67.5	94.7	0	6.7	33.3	97.5	0	24.7	51.3	158
84-22D	100.0	0	13.3	73.3	100.0	0	59.3	65.9	100.0	0	54.7	66.7	150
84-22F	-	-	-	-	99.3	0	50.7	79.3	99.3	0	50.7	79.3	150
All inoc	97.5	0	35.4	60.8	77.9	8.9	33.4	71.5	80.8	7.5	33.7	59.8	1,059
Control	-	-	-	-	14.7	11.3	28.7	70.0	14.7	11.3	28.7	70.0	150

[1]For inoculated seedlings, percentage of root samples from which the inoculated isolate was recovered; for control seedlings, percentage of root samples from which Fusarium oxysporum was recovered.

[2]For inoculated seedlings, percentage of root samples from which other (non-inoculated) Fusarium species were recoving; for control seedlings, percentage of root samples from which Fusarium (other than F. oxysporum) was recovered.

[3]Percentage of root samples from which Trichoderma spp. was recovered.

[4]Percentage of root samples from which Penicillium spp. was recovered.

Table 6.—Recovery of fungi from inoculated roots of lodgepole pine seedlings.

	PERCENTAGE OF ROOT SAMPLES												
	Dead seedlings				Live seedlings				All seedlings				
Isolate	Inoc[1]	Other[2] FUS	TRI[3]	PEN[4]	Inoc[1]	Other[2] FUS	TRI[3]	PEN[4]	Inoc[1]	Other[2] FUS	TRI[3]	PEN[4]	No. root samples
84-21A	100.0	0	80.0	0	35.8	0	18.8	27.9	49.3	0	23.9	25.6	180
84-21B	87.9	0	86.2	6.9	84.2	0	61.4	0	85.5	0	69.8	2.3	172
84-22A	92.1	28.5	62.3	11.3	-	-	-	-	92.1	28.5	62.3	11.3	151
84-22B	96.0	0	40.0	4.0	98.7	0	14.2	1.3	97.2	0	28.8	2.8	177
84-22C	99.0	0	66.7	0	98.3	0	45.0	6.7	98.7	0	58.3	0.6	156
84-22D	98.2	0	48.6	3.7	98.2	0	21.4	7.1	98.2	0	39.4	4.8	165
84-22F	100.0	0	41.1	0	96.6	0	35.6	0	98.8	0	41.9	0	161
All inoc	95.9	6.8	56.7	4.6	75.7	0	32.4	9.8	86.7	3.7	45.6	7.0	1,162
Control	0	0	70.0	0	22.5	0	57.0	10.6	19.8	0	58.6	9.3	162

[1]For inoculated seedlings, percentage of root samples from which the inoculated isolate was recovered; for control seedlings, percentage of root samples from which Fusarium oxysporum was recovered.

[2]For inoculated seedlings, percentage of root samples from which other (non-inoculated) Fusarium species were recoving; for control seedlings, percentage of root samples from which Fusarium (other than F. oxysporum) was recovered.

[3]Percentage of root samples from which Trichoderma spp. was recovered.

[4]Percentage of root samples from which Penicillium spp. was recovered.

Table 7.—Recovery of fungi from inoculated roots of western larch seedlings.

	PERCENTAGE OF ROOT SAMPLES												
	Dead seedlings				Live seedlings				All seedlings				
Isolate	Inoc[1]	Other[2] FUS	TRI[3]	PEN[4]	Inoc[1]	Other[2] FUS	TRI[3]	PEN[4]	Inoc[1]	Other[2] FUS	TRI[3]	PEN[4]	No. root samples
84-21A	100.0	0	0	47.1	24.1	0	7.1	20.6	32.3	0	6.3	23.4	158
84-21B	100.0	0	6.7	20.0	98.6	0	6.5	18.0	98.7	0	6.5	18.2	154
84-22A	100.0	12.8	12.8	40.4	-	-	-	-	100.0	12.8	12.8	40.4	141
84-22B	95.8	0	7.2	15.7	-	-	-	-	95.8	0	7.2	15.7	166
84-22C	100.0	1.5	4.6	1.5	86.5	1.1	16.9	20.2	91.6	1.3	11.7	12.3	154
84-22D	100.0	0.8	4.1	0.8	96.8	0	12.9	9.7	99.3	0.7	5.9	2.6	151
84-22F	-	0	2.2	14.3	100.0	0	5.0	21.7	100.0	0	3.3	17.2	152
All inoc	98.7	3.2	6.7	17.7	73.5	0.2	8.9	19.1	87.9	1.9	7.6	18.3	1,076
Control	10.5	21.0	10.5	26.3	23.2	0	15.5	44.4	21.7	3.3	14.9	42.2	161

[1]For inoculated seedlings, percentage of root samples from which the inoculated isolate was recovered; for control seedlings, percentage of root samples from which Fusarium oxysporum was recovered.

[2]For inoculated seedlings, percentage of root samples from which other (non-inoculated) Fusarium species were recoving; for control seedlings, percentage of root samples from which Fusarium (other than F. oxysporum) was recovered.

[3]Percentage of root samples from which Trichoderma spp. was recovered.

[4]Percentage of root samples from which Penicillium spp. was recovered.

Table 8.—Recovery of fungi from inoculated roots of blue spruce seedlings.

	PERCENTAGE OF ROOT SAMPLES												
	Dead seedlings				Live seedlings				All seedlings				
Isolate	Inoc[1]	Other[2] FUS	TRI[3]	PEN[4]	Inoc[1]	Other[2] FUS	TRI[3]	PEN[4]	Inoc[1]	Other[2] FUS	TRI[3]	PEN[4]	No. root samples
84-21A	60.0	13.3	20.0	53.3	45.9	32.6	27.4	54.8	47.3	30.7	26.7	54.7	150
84-21B	100.0	0	41.7	18.3	70.0	0	43.3	73.3	82.0	0	42.7	51.3	150
84-22A	93.9	23.1	51.7	44.2	26.6	0	73.3	60.0	87.6	21.0	53.7	45.7	162
84-22B	100.0	0	10.7	10.7	96.0	0	15.8	23.7	98.0	0	13.2	17.2	151
84-22C	100.0	0	11.9	14.3	100.0	0	37.7	75.5	100.0	0	30.4	58.1	148
84-22D	100.0	0	17.9	16.0	100.0	0	28.9	68.9	100.0	0	21.2	31.8	151
84-22F	100.0	0	6.1	21.0	100.0	0	22.2	42.2	100.0	0	19.7	33.5	152
All inoc	97.0	7.1	28.8	25.2	79.5	7.9	30.9	56.7	87.9	7.5	29.9	41.7	1,064
Control	-	-	-	-	15.3	0	14.7	64.7	15.3	0	14.7	64.7	150

[1]For inoculated seedlings, percentage of root samples from which the inoculated isolate was recovered; for control seedlings, percentage of root samples from which Fusarium oxysporum was recovered.

[2]For inoculated seedlings, percentage of root samples from which other (non-inoculated) Fusarium species were recoving; for control seedlings, percentage of root samples from which Fusarium (other than F. oxysporum) was recovered.

[3]Percentage of root samples from which Trichoderma spp. was recovered.

[4]Percentage of root samples from which Penicillium spp. was recovered.

Table 9.—Recovery of fungi from inoculated roots of all species of seedlings.

	PERCENTAGE OF ROOT SAMPLES												
	Dead seedlings				Live seedlings				All seedlings				
Isolate	Inoc[1]	Other[2] FUS	TRI[3]	PEN[4]	Inoc[1]	Other[2] FUS	TRI[3]	PEN[4]	Inoc[1]	Other[2] FUS	TRI[3]	PEN[4]	No. root samples
84-21A	87.2	4.2	31.9	34.0	35.2	13.4	16.1	43.3	39.0	12.7	17.2	42.6	638
84-21B	94.7	0	57.1	13.5	78.1	3.6	26.0	43.6	81.6	2.9	32.6	37.2	626
84-22A	95.2	21.6	42.8	31.7	77.0	12.7	74.5	84.8	90.2	19.2	51.5	46.2	604
84-22B	96.2	0	19.9	16.7	94.7	2.5	9.8	27.9	95.7	0.9	16.1	20.9	645
84-22C	99.3	0.3	37.1	22.0	94.8	0.3	26.4	37.6	96.9	0.3	31.3	30.4	616
84-22D	99.4	0.3	22.5	9.4	99.2	0	40.8	47.6	99.3	0.2	30.4	25.9	618
84-22F	100.0	0	22.3	10.2	99.2	0	33.4	47.3	99.5	0	28.8	31.9	614
All inoc	97.5	5.2	31.4	18.9	77.0	5.1	28.0	44.9	85.8	5.1	29.5	33.5	4,361
Control	-	10.3	41.0	12.8	18.8	2.9	28.8	47.9	18.0	3.4	29.5	45.7	623

[1]For inoculated seedlings, percentage of root samples from which the inoculated isolate was recovered; for control seedlings, percentage of root samples from which Fusarium oxysporum was recovered.

[2]For inoculated seedlings, percentage of root samples from which other (non-inoculated) Fusarium species were recovering; for control seedlings, percentage of root samples from which Fusarium (other than F. oxysporum) was recovered.

[3]Percentage of root samples from which Trichoderma spp. was recovered.

[4]Percentage of root samples from which Penicillium spp. was recovered.

Effects of the different Fusarium isolates on seed germination, time required for germination, germling mortality, and survival of germlings are summarized for Scots pine and blue spruce in tables 9 and 10, respectively. The only isolate that consistently reduced seed germination of both species was 84-22A (F. acuminatum). Time required for germination was generally not affected by any of the Fusarium isolates tested. All Scots pine germlings were killed by all tested isolates. In addition, mortality of noninoculated control germlings was also high. However, the survival time (days between seed germination and germling mortality) was reduced by all isolates, especially 84-22A. This indicates increased virulence for those isolates that infect, colonize, and kill germlings rapidly.

High rates of germling mortality were also evident for most blue spruce treatments. Average survival time was generally greater for blue spruce than Scots pine, and isolate 84-22A again displayed the greatest virulence on this basis.

Inoculated Fusarium isolates were recovered from all germlings (table 11). As with the larger seedling experiments, other isolates of Fusarium were recovered from some germlings, especially the noninoculated control treatments. Cross treatment inoculum movement as well as natural seed contamination may have been involved. Saprophytic species of Trichoderma and Penicillium were also recovered from several germlings. Consistent relationships between colonization of seedlings by these saprophtic fungi and infection by the Fusarium isolate tested were not evident.

Table 10.--Effects of Fusarium isolates on Scots pine seed germination and young germling mortality.

Isolate	Percent seed germination[1]	Avg. germination time[2]	Percent germling mortality[3]	Avg. survival time[4]
84-21A	93.3	13.1	100.0	19.1
84-21B	77.4	14.6	100.0	16.8
84-22A	64.5	13.0	100.0	7.4
84-22B	83.9	13.0	100.0	15.4
84-22C	80.0	13.5	100.0	13.1
84-22D	86.7	13.8	100.0	15.1
84-22F	77.4	12.4	100.0	18.3
All inoculated	80.4	13.3	100.0	15.3
Control	76.7	13.6	91.3	32.5

[1] From 30-31 seeds sown for each isolate; 30 for the control
[2] Number of days between sowing and germination (emergence of hypocotyl)
[3] Pecentage of germinated seedlings killed
[4] Number of days between germination and germling mortality

Table 11.--Effects of Fusarium isolates on blue spruce seed germination and young germling mortality.

Isolate	Percent seed germination[1]	Avg. germination time[2]	Percent germling mortality[3]	Avg. survival time[4]
84-21A	93.8	19.0	50.0	33.1
84-21B	86.7	18.4	92.3	24.1
84-22A	60.0	17.3	94.4	15.4
84-22B	83.3	19.9	88.0	23.5
84-22C	93.3	19.5	78.6	24.5
84-22D	93.8	17.6	80.0	25.4
84-22F	97.0	17.8	90.6	29.7
All inoculated	87.1	18.6	81.50	25.4
Control	97.1	18.8	36.4	33.3

[1]From 30-33 seeds sown for each isolate; 34 for the control
[2]Number of days between sowing and germination (emergence of hypocotyl)
[3]Pecentage of germinated seedlings killed
[4]Number of days between germination and germling mortality

Table 12.--Recovery of fungi from young inoculated Scots pine and blue spruce germlings.[1]

	Scots pine			Blue spruce		
Isolate	Other FUS[2]	TRI[3]	PEN[4]	Other FUS[2]	TRI[3]	PEN[4]
84-21A	17.9	3.6	0	33.3	0	20.0
84-21B	4.2	4.2	0	15.4	3.8	0
84-22A	20.0	0	0	44.4	11.1	16.7
84-22B	3.9	3.8	0	0	4.0	0
84-22C	0	0	0	0	0	10.7
84-22D	0	0	0	0	3.3	0
84-22F	12.5	0	0	0	0	0
All inoculated	8.1	1.7	0	11.6	2.6	6.2
Control	13.0/ 78.3	4.3	4.3	36.4/ 84.8	15.1	9.1

[1]Inoculated Fusarium isolates were recovered from all germlings.
[2]Other Fusarium (noninoculated) isolates; for control germlings figures represent percentages of germlings from which Fusarium oxysporum and other Fusarium species were recovered, respectively.
[3]Trichoderma spp.
[4]Penicillium spp.

Natural infection of blue spruce seed by F. oxysporum was high (table 2). However, this isolate was morphologically different from the isolates used for inoculation; i.e., it produced distinctive orange sporodochia on seed and in culture. Some of the "Other Fusarium" isolated from germlings (table 11) were probably F. oxysporum which contaminated seed. No Fusarium contaminants were located on the Scots pine seed sampled (table 12).

Table 13.--Occurrence of fungi on samples of Scots pine and blue spruce seed used for Fusarium inoculation experiments.

	Percent of sampled seed[1] with			
	Fusarium[2]	Trichoderma	Penicillium	Bacteria
Scots pine	0	3	74	17
Blue spruce	80	15	0	0

[1]100 seed from each species plated on Komada's medium (Komada 1975).
[2]Fusarium oxysporum with salmon-colored sporodochia.

Inoculum densities of the soil mixes used in seedling inoculations are summarized in table 13. The wide range of densities obtained may have been due to the aggregative nature of the cornmeal perlite inoculum as compared to other types of inoculum, such as spores. We would expect that spore inoculum would be more evenly distributed within soil mixes and analyses would therefore yield more uniform density estimates.

Table 14.--Inoculum density of Fusarium isolates used to inoculate ponderosa pine, lodgepole pine, western larch, and blue spruce seedlings.[1]

	Species				
Isolate	Ponderosa pine	Lodgepole pine	Western larch	Blue spruce	Average
84-21A	0	2,900	4,700	3,300	2,725
84-21B	2,000	600	8,900	2,400	3,475
84-22A	1,300	1,900	0	1,600	1,200
84-22B	13,400	0	4,700	0	4,525
84-22C	900	900	1,700	3,200	1,675
84-22D	900	14,500	1,300	300	4,250
84-22F	5,100	0	800	400	1,575
Control	0	0	0	0	0

[1]Expressed as propagules per gram of soil mixture.

DISCUSSION

The most common Fusarium species obtained from the Alpine Nursery was F. oxysporum. This species is a common soil inhabitant that causes diseases of many different types of plants including conifer seedlings (Booth 1971; Edmonds and Heather 1973). Because most forms are similar morphologically differentiation among the species "F. oxysporum" is often made on the basis of host pathogenicity (Armstrong and Armstrong 1975; Gordon 1965). Pathogenic and saprophytic strains cannot usually be distinguished any other way. Isolates pathogenic to specific hosts are designated within a formae specialis (f. sp.). For example, those that attack tomato are designated F. oxysporum Schlect f. sp. lycopersici (Sacc.) S. & H. The forms that attack conifer seedlings are generally grouped together in f. sp. pini (Hartig) S. & H (Gordon 1965; Snyder and Hansen 1940). The ability of isolates within this f. sp. to attack several different conifer hosts is unclear; only a few multi-host pathogenicity tests have been reported. In one such study (Matuo and Chiba 1966), two races of F. oxysporum f. sp. pini were separated on their ability to kill (cause damping-off) different conifer species. Race designations were proposed even though the tested isolates attacked all host species. However, the severity of attack, as indicated by percentage of seedlings killed, varied among the different isolates. In our experiments, only two isolates of F. oxysporum (84-22B and 84-22C) consistently killed ponderosa pine. This differential pathogenicity of the tested isolates to ponderosa pine may indicate race differences. However, we believe more pathogenicity tests conducted using a greater number of isolates and host species are necesary to confirm apparent pathogenic differences. If consistent pathogenic differences exist, new races or additional formae specialis may be assigned to F. oxysporum affecting conifer seedlings.

Another Fusarium species obtained from the Alpine Nursery was F. acuminatum. This species has worldwide distribution and occurs on many different hosts (Gerlach and Nirenberg 1982). Fusarium acuminatum has been reported causing damping-off of conifer seedlings (Vaartaja and Cram 1956) and alfalfa seedlings (Hancock 1983), and a foot and root rot of legumes (Booth 1971). The isolate we tested was the most virulent of all Fusarium isolates evaluated, except on pondersa pine.

The other Fusarium species obtained from the Alpine Nursery was F. sambucinum. Virulence of the F. sambucinum isolate we tested was generally low, i.e., few seedlings were killed and its ability to cause damping-off of young germlings was limited. Fusarium sambucinum causes three types of diseases: cankers on woody plants, root rots, and storage rots (Booth 1971). As a root and seedling rot, the fungus has been reported mostly from eastern Europe and USSR, attacking seedlings of cereals, forest trees, lupin, tomato, and strawberry (Booth 1971). Apparently, isolates that cause a particular type of disease cannot cause other types of disease.

Occurrence of virulent Fusarium isolates in soil mixes was unexpected because commercially prepared mixes used for growing conifer seedlings are generally pathogen-free (James 1984; Tinus and McDonald 1979). However, if soil mixes become contaminated, aggressiveness of pathogens, particularly Fusarium species, can be limited if the pH is kept below 6.0 (Pawuk 1981). High pH values (6.0-6.7) and the sizable Fusarium populations in the soil mixes probably contributed to the extensive seedling losses that occurred at the Alpine Nursery.

These experiments indicated that, in most cases, the techniques of inoculum production and seedling inoculation were successful in quantitatively differentiating pathogenic potential of the Fusarium isolates tested. Inoculum densities may have been excessive for some tests, especially the Scots pine seed germination tests where all germlings were killed by each isolate. Threshold levels of soil inoculum required to initiate disease vary according to several environmental conditions (Edmonds and Heather 1973; Manandhar and Bruehl 1973; Tint 1945b). Therefore, it is difficult to predict the amount of inoculum necessary for pathogenicity screening. Since all inoculated seedlings became infected in our tests even though some lacked foliage symptoms, we suspect our inoculum densities were sufficient. Tests to monitor responses of several host species to different inoculum densities using standard growing conditions would be beneficial.

Problems encountered in these experiments included apparent cross-contamination of inoculum in some tests and the requirement for more refined estimates of root infection. Cross-contamination was especially apparent in several noninoculated controls. Such contamination could have been prevented with greater spacial separation of the various treatments. However, due to limited growth chamber capacities and the fact that different treatments were conducted simultaneously (to help reduce variation), some cross-contamination occurred. To obtain more refined estimates of root infection, the techniques of Schneider (1984) and Schneider and Pendery (1983) could be used in future tests. These techniques allow more consistent root infection estimates that might help elucidate quantitative relationships between foliage symptoms and root infection. Quantitative root infection estimates could also help define inoculum density-host response relationships.

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APPENDIX

Characteristics of *Fusarium* Isolates From the Alpine Nursery

84-21A (Spruce seedling)

Tentative Identification: *Fusarium sambucinum* Fuckel (= *F. roseum* Link emend. Snyd. & Hansen). Teleomorph: *Gibberella pulicaris* (Fr.) Sacc.

Charactierstics on PDA.
Characteristics on PDA

1. Colonies fast growing, reaching 8 cm in diameter in 6 days at 22°C.
2. Colonies with floccose pink to light-orange aerial mycelium (figure 3).
3. Sporulation common in culture; however, distinct sporodochia not common in young (14 days) cultures.
4. Only macroconidia formed, mostly 3-5 septate, compact, dorsiventrally curved, with short attenuate and constricted apical and distinctly pedicellate basal cells.
5. Intercalary, chlamydospores present (figure 4), often formed in pairs with a brownish pigmentation.

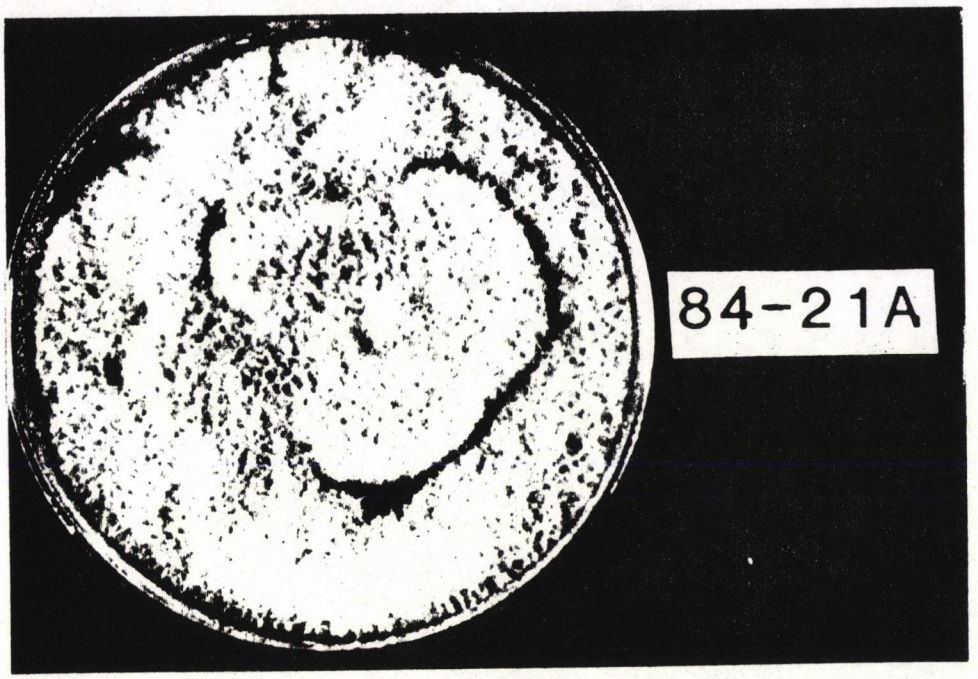

Figure 3.--Isolate 84-21A (*F. sambucinum*) on PDA after 10 days at 22°C.

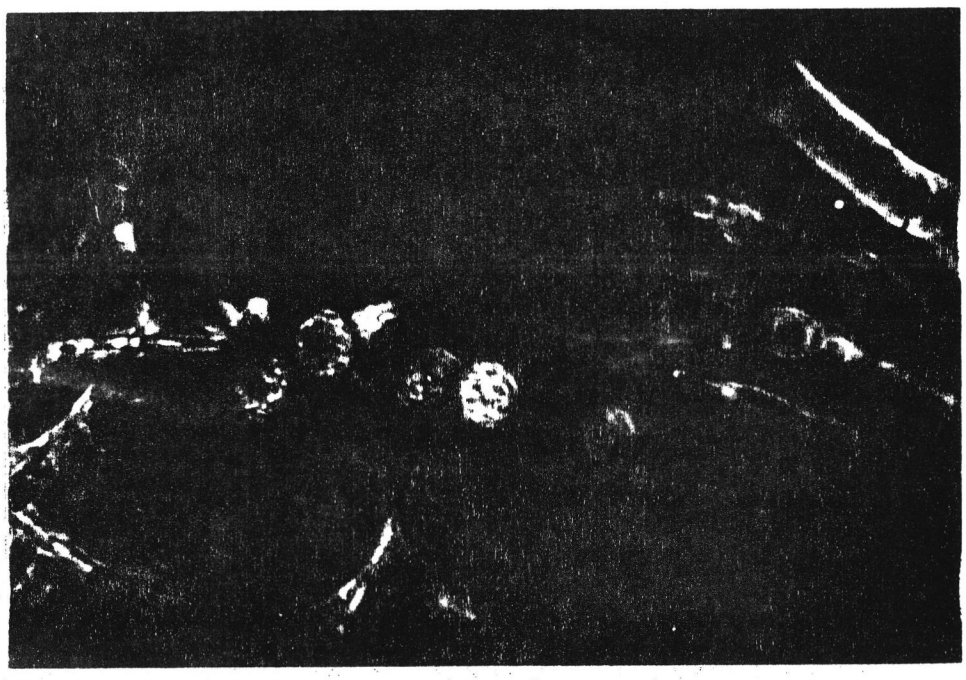

Figure 4.--Chlamydospores of isolate 84-21A (F. sambucinum) produced on PDA. Chlamydospores were produced mostly in chains and were intercalary (X450).

84-21B (Spruce seedling)

Tentative Identification: Fusarium oxysporum Schlect.

Characteristics on PDA

1. Colonies moderately fast growing, reaching 6.5 cm in diameter in 7 days at 22°C.

2. Colonies at first with white to light violet floccose mycelium. As cultures age, mycelium becomes appressed and slimy (figure 5) and violet pigmentation intensifies.

3. Abundant, mostly one-celled cylindric to ellipsoid microconidia formed in groups on short monophialides.

4. Abundant falcate macroconidia, 3-5 septate, with distinct pedicellate basal cells and a beaked apex produced.

5. Sporodochia are not produced, although purple plectenchymatous sclerotia are produced in older cultures. Distinct, mostly terminal chlamydospores evident in cultures several weeks old.

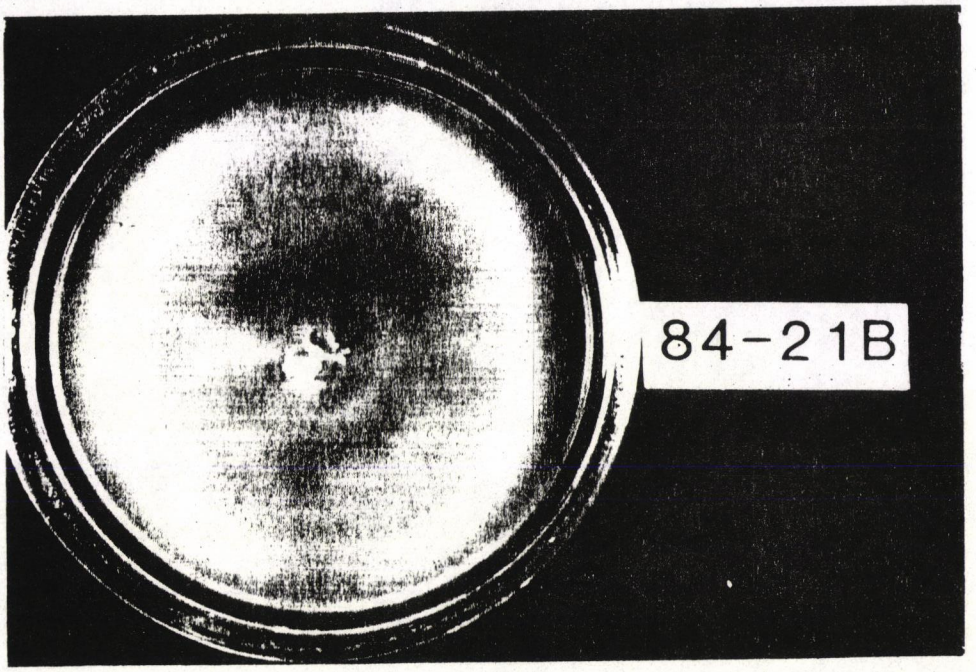

Figure 5.--Isolate 84-21B (F. oxysporum) on PDA after 14 days at 22°C.

84-22A (Soil mix - benches 8 and 9)

Tentative Identification: Fusarium acuminatum Ell. & Kellerm. (= F. roseum Link emend. Snyd. & Hansen). Teleomorph: Gibberlla acuminata Booth.

Characteristics on PDA

1. Colonies fast growing, reaching 8 cm in diameter in 5 days at 22°C.

2. Colonies at first whitish to rose color becoming more pink with age. Colony margin uneven and lobed (figure 6).

3. Sporulation common in culture on PDA and starting in young cultures. Sporulation occurs on aerial mycelium; sporodochia not produced.

4. Macroconidia falcate, 3 to 5 septate, equilaterally curved, slender, mostly with a moderately elongated, pointed apical cell, and a distinctly pedicellate basal cell. Some macroconidia small with 0-2 septations (may be developing into larger conidia).

5. Chlamydospores abundantly produced, mostly intercalary, globose to subglobose, and often in chains.

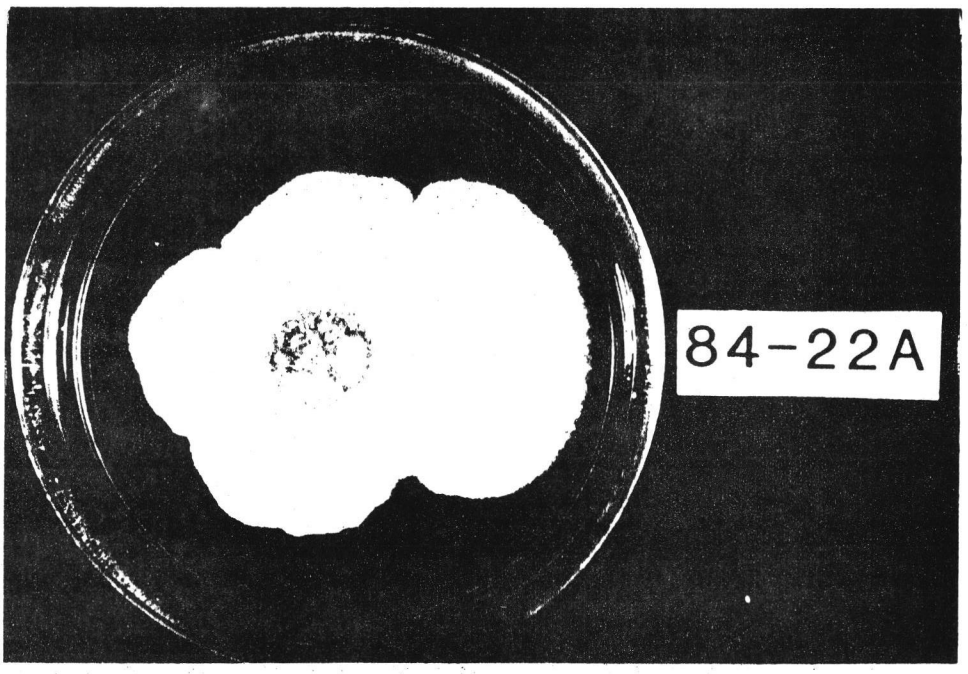

Figure 6.--Isolate 84-22A (F. sambucinum) on PDA after 5 days at 22°C.

84-22B (Soil mix - benches 3 and 4)

Tentative Identification: Fusarium oxysporum Schlect.

Characteristics on PDA

1. Colonies fast growing, reaching 7 cm in diameter in 7 days at 22°C.

2. Colonies with white floccose mycelium producing a slight violet pigment (figure 7). With age, mycelium becomes appressed and slimy, and violet pigment intensifies. Cream colored to slightly orange sporodochia sparsely produced in cultures several weeks old; no sclerotia produced.

3. Abundant, mostly single-celled, cylindric to ellipsoid microconidia formed in groups on short, unbranched monophialides.

4. Macroconidia falcate, 3-5 septate, with distinct pedicellate basal cells and a beaked apex.

5. Chlamydospores, sparsely formed in cultures several weeks old, are mostly globose to subglobose and produced terminally on hyphae.

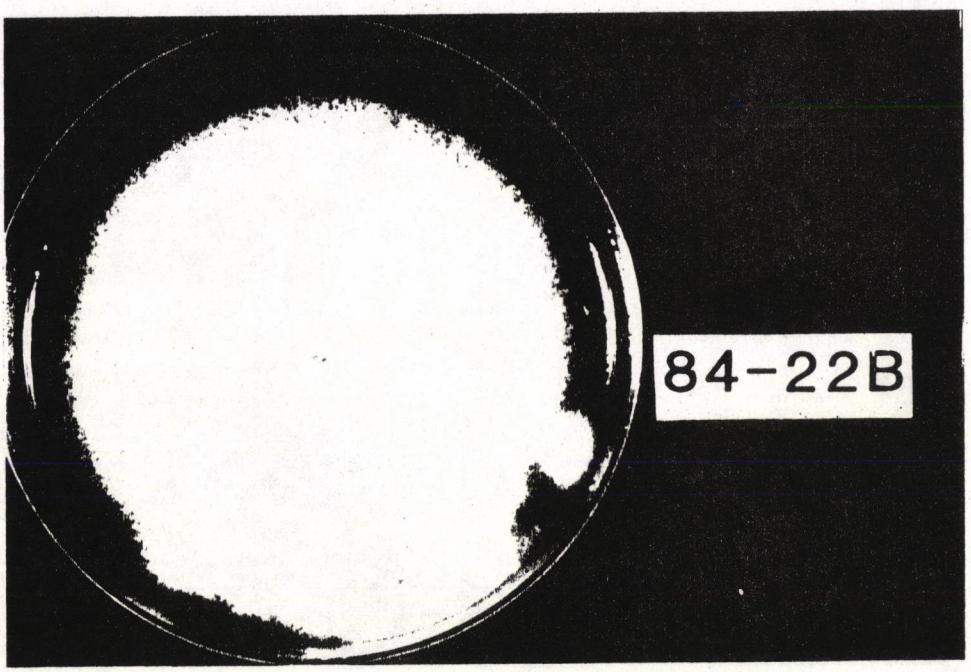

Figure 7.--Isolate 84-22B (F. oxysporum) on PDA after 10 days at 22°C.

84-22C (Soil mix - bench 2)

Tentative Identification: Fusarium oxysporum Schlect.

Characteristics on PDA

1. Colonies, fast growing, reach 8.5 cm in diameter in 7 days at 22°C.

2. Colonies with white floccose mycelium producing a slight violet pigment (figure 8). With age, mycelium becomes appressed but the violet pigment does not intensify. No sclerotia or sporordochia produced.

3. Abundant, mostly single-celled, cylindric to ellipsoid microconidia formed in groups on short, unbranched monophialides.

4. Macronidia falcate, 3-5 septate, with distinct pedicellate basal cells and a beaked apex.

5. Chlamydospores, formed in cultures several weeks old, are mostly globose to subglobose and produced terminally on hyphae.

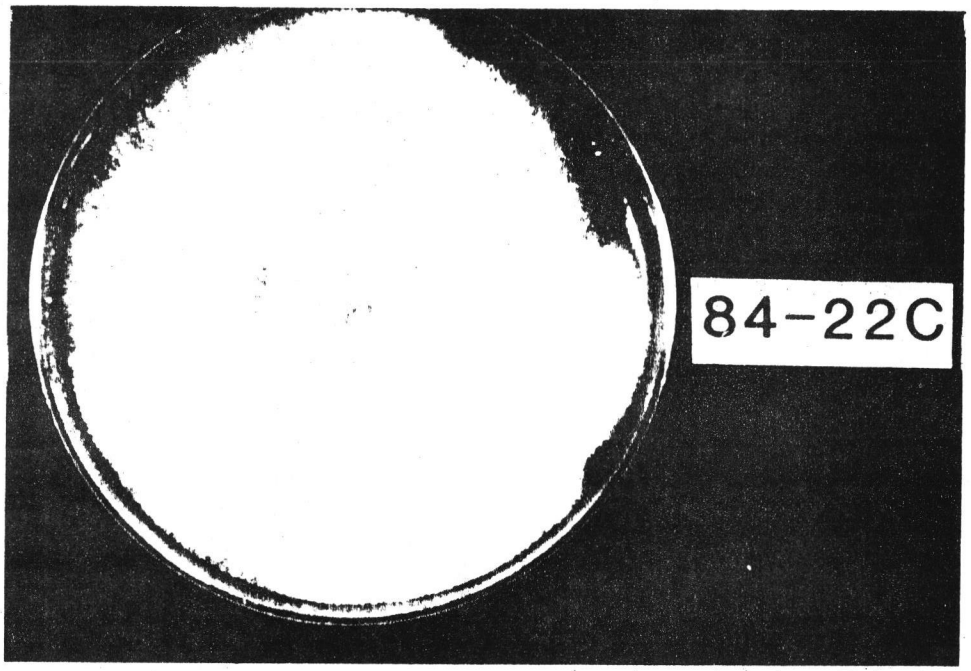

Figure 8.--Isolate 84-22C (F. oxysporum) on PDA after 10 days at 22°C.

84-22D (Soil mix - benches 5 and 6)

Tentative Identification: Fusarium oxysporum, Schlect.

Characteristics on PDA

1. Colonies, fast growing, reaching 8 cm in diameter in 7 days at 22°C.

2. Colonies with white floccose mycelium producing violet pigment (figure 9). As colonies age, pigmentation intensifies and distinct violet-black plectenchymatous sclerotia are formed.

3. Sporulation abundant in young cultures; cream-colored sporodochia may be produced in older cultures.

4. Abundant single- or two-celled cylindric to ellipsoid microconidia borne on short, unbranched monophialides (figures 10 and 11).

5. Macroconidia less common, 3-5 septate with distinct pedicellate basal cells (figure 11). Chlamydospores mostly terminal, occasionally intercalary, and globose to subglobose.

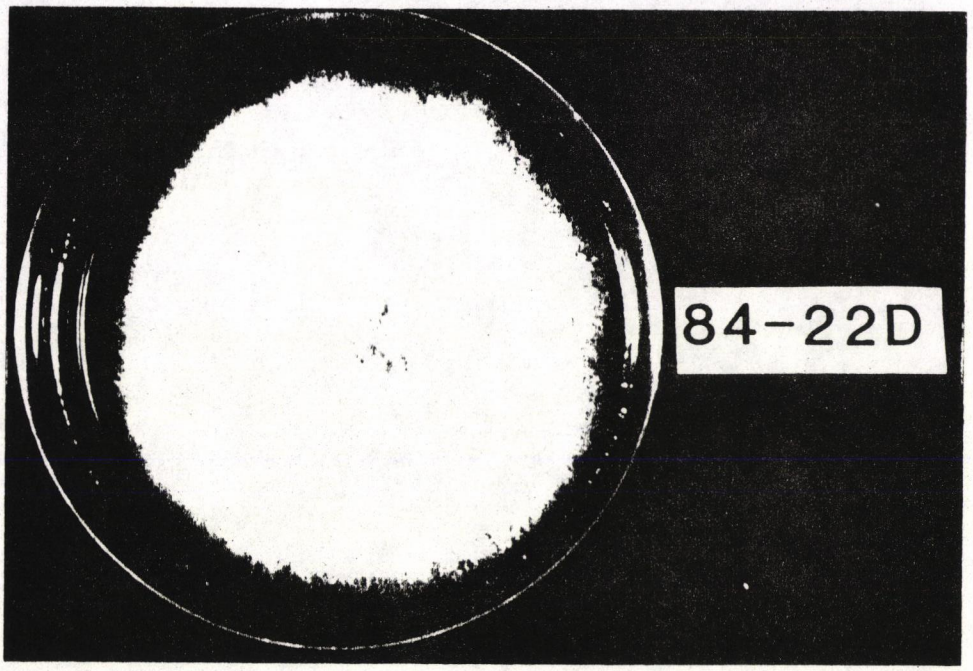

Figure 9.--Isolate 84-22D (F. oxysporum) on PDA after 10 days at 22°C.

Figure 10.--Groups of microconidia borne on short unbranched monophialides (arrows) in isolate 84-22D (F. oxysporum) grown on PDA (X450).

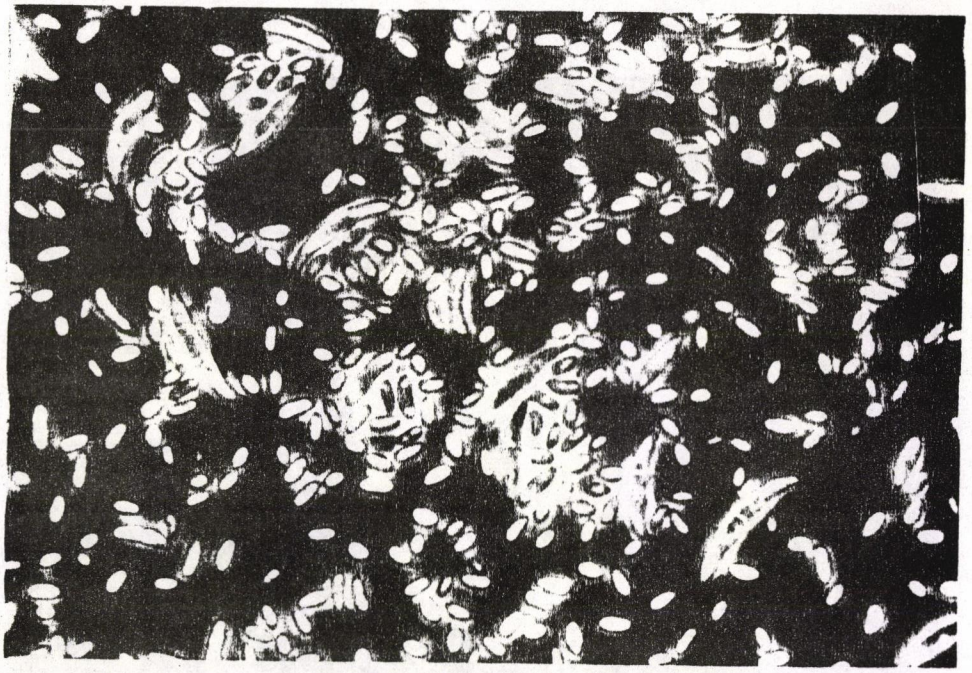

Figure 11.--One- and two-celled cylindric to ellipsoid microconidia (black arrow) and 3-5 septate falcate macroconidia (red arrow) of isolate 84-22D (F. oxysporum grown on PDA (x450)).

84-22F (Soil mix - spruce seedling)

Tentative Identification: Fusarium oxysporum Schlect.

Characteristics on PDA

1. Colonies moderately fast growing, reaching 6.5 cm in diameter in 7 days at 22°C.

2. Colonies at first with white to light violet floccose mycelium (figure 12). As cultures age, mycelium becomes appressed and violet pigmentation intensifies.

3. Abundant mostly one-celled cylindric to ellipsoid microconidia formed in groups on short monophialides.

4. Abundant falcate macroconidia, 3-5 septate with distinct pedicellate basal cells and a beaked apex.

5. Sporodochia not produced, although purple plectenchymatous sclerotia in older cultures. Distinct, mostly terminal chlamydospores evident in cultures several weeks old.

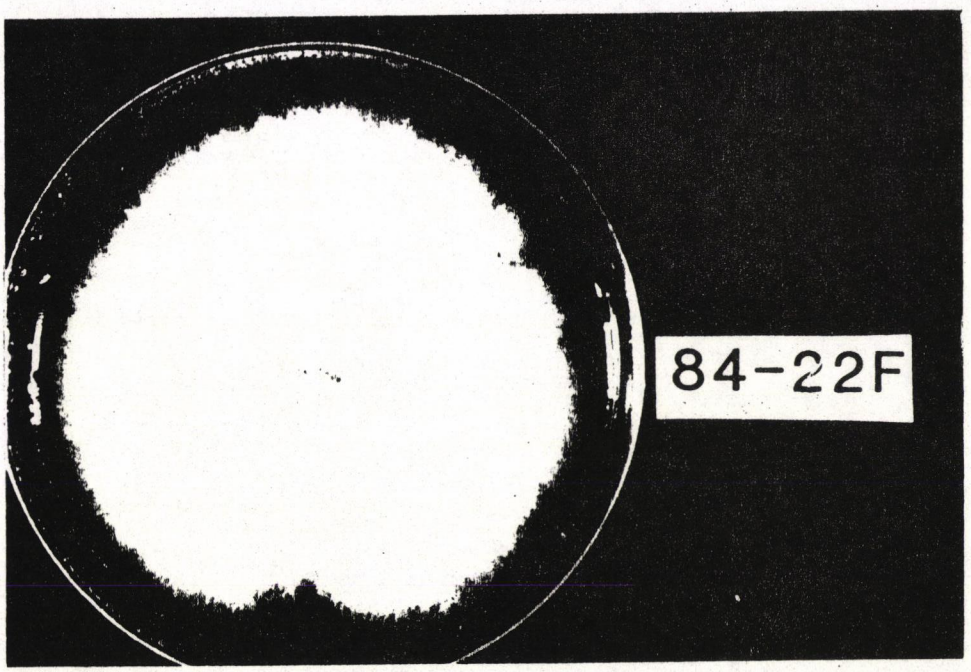

Figure 12.--Isolate 84-22F (F. oxysporum) on PDA after 10 days at 22°C.